REMARKS

Claims 26-50 are pending in the present application. Claim 41 is amended to correct the obvious misspelling of "antiangiogenic." No new matter is added. Pursuant to the following remarks, Applicants respectfully request reconsideration of the application and allowance of the claims to issue.

Rejections Under 35 U.S.C. § 112, first paragraph

A. The Office Action states that claims 26-50 are rejected under 35 U.S.C. § 112, first paragraph, because the specification while being enabling for the methods set forth on pages 3-5 of the Office Action allegedly does not reasonably provide enablement for measuring angiogenic or anti-angiogenic activity of a test molecule using any other substrate or fluorescent labeled particle or assigning any brightness to each pixel in the image for calculating FVD or using any agent to measure metabolic activity at any other wavelength. The Examiner then goes on to provide an *in re Wands* analysis of the claims. Further stated on page 9 of the Office Action is that the "[p]rior art teaches that angiogenesis is the growth of new vascular capillary channels from pre-existing vessels, and is of fundamental importance in a number of physiological processes such as embryonic development, reproduction, wound healing and bone repair. Uncontrolled angiogenesis is pathological and is often associated with tumor growth, rheumatoid arthritis, diabetic retinopathy and hemangiomas (Folkman et al. J. Biol. Chem. 1992; 267(16): 10931-4). It is also noted that a number of methods are currently being used to measure angiogenic and anti-angiogenic activity. Munoz-Chapuli et al. (Cell Mol. Life Sci. 2004: 61(18): 2224-43) describe 'angiogenic signals promote endothelial cell proliferation, increased resistance to apoptosis, changes in proteolytic balance, cytoskeletal reorganization, migration and, finally differentiation and formation of a new vascular lumen (see abstract).'"

Also stated in the Office Action is that the "...prior art teaches the limitations of using CAM assay for measuring angiogenic or angiogenic activity. For instance, Ribatti et al. (The Anatomical Record 2001, 264, 317-324) state, 'main limitation of CAM assay

is the nonspecific inflammatory response including a secondary vasoproliferative response and impending quantification of the primary response.' Ribatti et al. further teach that other drawback of CAM assay is that test molecule is placed in the existing vessel and newly formed vessel grow within the CAM mesenchyme, thus the real neovascularization can hardly be distinguishable from a falsely increased vascular density due to the rearrangement of existing vessel that follows the contraction of the membrane (see page 321, col. 2, para. 1)." According to the Office Action, Ribatti et al. also emphasized that the timing of the CAM angiogenic response is essential and noted that early angiogenesis after 24 hours only measures vasodilation and not the angiogenesis. Thus, the Office Action states that CAM itself is undergoing rapid changes both morphologically and in terms of the gradual change in the rate of endothelial cell proliferation during the course of embryonic development. Therefore, according to the Office Action, any measurement of fluorescence or metabolic product formation of vessel comprising cells by using a fluorescent particle or XTT may provide a result that may be because of vasodilation and not because of angiogenesis or because of changes in embryonic development. Further stated in the Office action is that the claims as recited do not recite a specific stage of egg being used for conducting CAM assay which would further mask the effect thereby resulting in fluorescent or absorbance reading that would not correctly reflect the angiogenic or antiangiogenic activity as contemplated by the specification. Thus, the Office Action concludes that an artisan of skill in the art would have to perform undue experimentation to practice the method as claimed because the art of measuring angiogenic activity in CAM assay using any fluorescence labeled particle or any agent having metabolic activity was allegedly unpredictable at the time of filing of this application.

With regard to the Examiner's allegation that the specification does not reasonably provide enablement for measuring angiogenic or anti-angiogenic activity of a test molecule using any other substrate or fluorescent labeled particle or assigning any brightness to each pixel in the image for calculating FVD, Applicants respectfully disagree and provide the following remarks in support of the enablement of the claims.

Although Applicants have exemplified the use of a filter disk as a substrate, on page 6, paragraph 16, of the specification, Applicants have described other suitable substrates, including, but not limited to, glass, plastic, nylon, silicon, polytetrafluoroethylene, Matrigel, collagen, fibrinogen, agarose and methylcellulose. Applicants respectfully point out that these substrates are included in claim 28. The Examiner has admitted that claim 28 has written description, which requires evidence of possession. Therefore, this claim is also enabled and its rejection for lack of enablement is improper. The claimed methods can use known substrates, such as those listed in claim 28, which are enabled, so those of skill in the art will know the relevant structures or source of those substrates. The claims require a substrate that can be applied to a test region of the CAM such that after administration of a test molecule and a fluorescent-labeled particle to a vessel in the CAM, the substrate and test region can be removed for capturing an image of the test region of interest. All that is required is a substrate that will physically associate with the test region of interest to allow removal of the test region of interest for subsequent analysis and image capture. Therefore, it would be routine for one of skill in the art to identify other substrates with these properties. For example, following the protocol taught/demonstrated in the Examples of the specification, one of skill in the art can provide a substrate to a test region of the CAM, administer a test molecule to a vessel in the CAM, administer a fluorescent-labeled particle to a vessel in the CAM, remove the substrate and the test region of the CAM, and capture a three dimensional image of the test region of interest. If the substrate associates with the test region of interest in the CAM such that upon removal of the substrate with the test region of interest, both the substrate and the test region of interest can be prepared, for example, by mounting both the substrate and the test region of interest on a glass slide, and suitable three dimensional images of the test region of the CAM can be captured and subsequently analyzed, one of skill in the art would know that the substrate is a suitable substrate. This can be done for any substrate now known or identified in the future. There is no basis asserted or believed that this testing would be difficult (other than routine) in nature or substantial in amount. However, even if one were to argue that

the testing of individual substrates may be extensive, Applicants remind the Examiner that this experimentation would be routine for one of skill in the art and not undue.

The Examiner has also indicated that the claims are allegedly not enabled for the use of any fluorescent particle. Applicants respectfully point out that those of skill in the art routinely use fluorescently labeled particles to analyze biological processes. The claimed methods use known fluorescent particles, so those of skill in the art will know the relevant structures or source of the fluorescent particles. On page 12, paragraph 0036 of the specification, Applicants have described numerous particles that can be associated with fluorescent moieties, such as, a carbohydrate, a protein, a polypeptide, a peptide. As stated on page 12, paragraph 0036 of the specification, "[a]ttachment of a particular fluorescent moiety to a particular particle is well known in the art and described in, for example, Haugland, RP, 'Coupling of Monoclonal Antibodies with Fluorophores,' in *Methods in Molecular Biology*, Vol. 45: Monoclonal Antibody Protocols, W.C. Davis, ed. (Totowa, NJ: Humana Press, 1995), pp.205-221." Thus, it would be routine for one of skill in the art to obtain a fluorescent labeled particle, for example, from a commercial source, or by attaching a fluorescent moiety to a particle of interest. It would also be routine for one of skill in the art provide a substrate to a test region of the CAM, administer a test molecule to a vessel in the CAM, administer a fluorescent-labeled particle to a vessel in the CAM, remove the substrate and the test region of the CAM, and capture a three dimensional image of the test region of interest. This protocol is taught in the Examples, such that the only variable is the particle. If the fluorescent labeled particle allows suitable image capture such that the angiogenic or antiangiogenic processes can be visualized and analyzed, one of skill in the art would know that the fluorescently labeled particle is suitable. This can be done for any fluorescent-labeled particle now known or identified in the future. There is no basis asserted or believed that this testing would be difficult (other than routine) in nature or substantial in amount. However, even if one were to argue that the testing of individual fluorescent-labeled particles may be extensive, Applicants remind the Examiner that this experimentation would be routine for one of skill in the art and not undue.

With regard to the Examiner's allegation that Applicants have not enabled assigning any brightness to each pixel in the image for calculating FVD, it is very standard for this type of measurement to use thresholding. In the present method, changes in brightness assignments are based on deviations from a set of controls (positive and negative) for each experiment. There is enough variability in the quantification of fluorescence that one usually uses "standards" for such assays (i.e., ELISA assays). It is not reasonable to set absolute values in a method such as that claimed. One of skill in the art would recognize this, and would view the assignment of brightness as a routine aspect of the type of assay covered by the claims.

The teaching of how to do this is explicit in the application. For example, Applicants direct the Examiner's attention to page 17, paragraph 0055 of the specification where Applicants describe how to calculate the FVD values of a test region of interest. Thus, it would be clear to one of skill in the art how to assign brightness values to each pixel, for example, a brightness value from 0 to 255, in order to obtain an FVD value for a test region of interest. Similarly, one of skill in the art can apply the same methodology described on page 17, paragraph 0055 of the specification with other brightness value ranges in order to obtain an FVD value for a test region of interest. The Examiner has not stated a reason why Applicant's teaching should not be taken at face value, as required to support an enablement rejection. In fact, the specification and the art both teach that such experimentation would be routine for one of skill in the art.

Regarding the use of agents to measure metabolic activity at any other wavelength, Applicants respectfully point out that XTT is merely an example of an agent that can be utilized to measure metabolic activity at 450-500 nm. On page 20, paragraph 0065, Applicants have also described MTT (550-600 nm) and WST-1 (420-480 nm), both available from Roche Applied Science as other agents that can be used to measure metabolic activity. The art is replete with additional examples of fluorescent particles and their excitation wave lengths. It would be clear to one of skill in the art, upon reading the specification that once Applicants have shown that XTT can be utilized in the claimed methods, numerous agents can be utilized at different wavelengths to measure metabolic activity. For example, one of skill in the art can substitute MTT for XTT and

utilize a wavelength of 550-600 nm to measure metabolic activity. One of skill in the art can also substitute WST-1 for XTT and utilize a wavelength of 420-480 nm to measure metabolic activity. Thus, it is clear that these agents and the wavelengths at which their reduction products can be measured by spectrophotometrical absorbance are known in the art and widely available. Therefore, it would be routine for one of skill in the art to utilize any similar agent at the appropriate wavelength to achieve the effects obtained by Applicants.

In response to the Examiner's characterization of the prior art, Applicants agree with the Examiner that a number of methods are currently being used to measure angiogenic and anti-angiogenic activity. Applicants also agree that the CAM assays previously described for measuring angiogenic or anti-angiogenic activity have limitations. In fact, the CAM assays described in Ribatti et al. utilize a stereomicroscope to count the number of blood vessel branch points within the CAM, which may not provide accurate results. The Examiner has acknowledged that the presently claimed methods are novel. Therefore, the observations made by others regarding previously described CAM assays should not apply to the presently claimed methods. In fact, as evidenced in Comparative Example 1, on pages 21-22 of the specification, it is Applicants' invention that provides a CAM assay that is superior to previous detection methods in the art. As indicated by the results set forth in Tables 2-4, when filter discs were evaluated by blinded observers using a detection method of the prior art, none of the three independent, blinded observers could visually appreciate differences between the groups when the methodology of blinded grading was applied. However, as indicated in Example 1, differences were seen when an FVD value was assigned to each group. Thus, Applicants have provided novel methods of measuring angiogenic and antiangiogenic activity that significantly improve the quantitation of these activities.

According to the Examiner Ribatti et al. teaches that a drawback of the CAM assay is that a test molecule is placed in the existing vessel and newly formed vessels grow within the CAM mesenchyme, thus the real neovascularization can hardly be distinguishable from a falsely increased vascular density due to the rearrangement of existing vessels that follows the contraction of the membrane. Applicants question the

basic premise of this rejection. It appears that a re-arrangement of existing vessels would not be interpreted as neo-vascularization, because the amount of fluorescence would not increase in the case of re-arrangement, but would increase in the case of neo-vascularization. Furthermore, the present method avoids this concern by assaying after the treatment effects have taken place on the vessels and fixing them following systemic administration of the fluorescent agent, using the confocal approach, which can examine the 3-D architecture of the vasculature. Applicants have also shown that the MTT or XTT version of the assay correlates with the confocal values.

As stated above, Ribatti et al. also emphasized that the timing of the CAM angiogenic response is essential and noted that early angiogenesis after 24 hours only measures vasodilation and not the angiogenesis. The present method overcomes this by assaying after the treatment effects have taken place on the vessels and fixing them following systemic administration of the fluorescent agent, using the confocal approach, which can examine the 3-D architecture of the vasculature.

For the reasons set forth above, Applicants believe that claims 26-50 are adequately enabled for measuring angiogenic or anti-angiogenic activity of a test molecule. Thus, Applicants believe this rejection has been overcome and respectfully request its withdrawal.

B. The Office Action states that claims 26-27, 29-41 and 43-50 are rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the written description requirement. In particular, the Office Action alleges that the specification does not describe the complete structure or any other physical or chemical specific structure or domain that are essential for a particle that would be suitable for labeling any fluorescent moiety or any other metabolic agent to measure angiogenic activity.

Applicants respectfully point out that the mere absence of some specific description in a specification of some embodiment or aspect of a claimed invention does not, by itself, constitute a lack of adequate written description. Rather, the test is that the applicant conveys with reasonable clarity to those of skill in the art that he or she invented what is claimed. Vas-Cath v. Mahurkar, 19 USPQ2d 1111, 1116 (Fed. Cir.

1991). It is clear that every detail of every embodiment is not required. For example, if subject matter is referred to in the specification as being part of the invention, and if that subject matter is not new or unknown subject matter that ordinarily skilled artisans would easily miscomprehend, then such subject matter is adequately described as required by 35 U.S.C. § 112, first paragraph. See Amgen v. Hoechst, 314 F.3d 1313, 1332 (Fed. Cir. 2003).

In Amgen, the claims of Amgen's patents referred to types of cells that can be used to produce recombinant human EPO. TKT (Amgen's opponent) argued that, because the Amgen patents did not describe the structure of the claimed cells, the patents failed to provide adequate written description of the claimed subject matter as required by Regents of the University of California v. Eli Lilly, 119 F.3d 1559 (Fed. Cir. 1997) and Enzo Biochem. v. Gen-Probe, 296 F.3d 1316 (Fed. Cir. 2002). The court in Amgen rejected this argument, holding that Amgen's claims, including the recited cells, were adequately described in Amgen's patents. The court noted that unlike in Eli Lilly or Enzo

> the claim terms at issue here [in Amgen] are not new or unknown biological materials that ordinarily skilled artisans would easily miscomprehend....This difference alone sufficiently distinguishes Eli Lilly, because when used, as here, merely to identify types of cells (instead of undescribed, previously unknown DNA sequences), the words 'vertebrate' and 'mammalian' readily 'convey distinguishing information concerning [their] identity' such that one of ordinary skill in the art could 'visualize or recognize the identify of the members of the genus.'

Amgen at 1332. Like the cells of Amgen, the substrates, fluorescent labeled particles and metabolic agents utilized in the claimed methods are well known biological materials, easily recognized by those of skill in the art. In this regard, and as in Amgen, the present claims make use of known materials in a new combination and used in a new way. The components utilized in the claimed methods themselves are not new or unknown materials that those of skill in the art would easily miscomprehend. As a result, and as in Amgen, the present application satisfies the written description requirement for the present claims. Because the present rejections fail to correctly apply the law of

written description the present claims, the rejections fail to establish a prima facie case of lack of adequate written description. For at least this reason, the present rejections should be withdrawn.

The rejections also fail to establish how it is that a lack of specific description of more embodiments of the substrate, the fluorescent labeled particle and the metabolic agent could constitute a lack of adequate written description. The rejections, although lengthy, consist almost entirely of statements regarding what is encompassed by the claims, what is specifically described in the specification, comparison of what is claimed and what is described in the specification, and conclusions (that are supported only by the statements and comparison) that the specification lacks adequate written description. With respect, more than this is required. The rejections must provide reasons why those of skill in the art would find the written description inadequate; that is, why they would not recognize that Applicants invented what is claimed. Reference in the specification to known materials, even in the absence of more specific description of numerous embodiments of such materials, would not constitute a lack of adequate written description because those of skill in the art would not miscomprehend that nature and use of such known materials. This is not a situation where, as in Eli Lilly, Applicants are claiming new and unknown components. Because the present rejections fail to support the conclusions drawn in the present rejections, the rejections fail to establish a prima facie case of lack of adequate written description. For at least this reason, the present rejections should be withdrawn.

Rejections Under 35 U.S.C. § 112, second paragraph

The Office Action states that claims 37-40 and 41-50 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite.

A. According to the Office Action, the term "relative fluorescent brightness" in claim 39 is a relative term that allegedly renders the claim indefinite. Further stated in the

Office Action is that it is allegedly unclear relative to what fluorescent brightness of each pixel would facilitate calculation of an FVD value.

Page 17 of the specification describes how to assign a brightness value, apply thresholding, etc. to obtain an FVD value. As noted above, this is a routine procedure in methods using this technology. Also, it is noted that there are numerous examples of "fluorescent brightness" and "relative fluorescence" in the literature, and examples of "brightness" and "relative fluorescence" in the same abstract, clearly relating these terms. Examples of abstracts from prior to applicants filing date are provided as Exhibits A-D. Exhibit E is post filing, but provides more recent confirmation that the term "relative fluorescence brightness" has recognized meaning in the art. Because these terms have understood meaning, and the term "relative fluorescent brightness" is nothing more than a contraction of these terms, the evidence now of record supports a conclusion that "relative fluorescence brightness" has a definite and well-understood meaning in the art. In the absence of contradictory evidence, the Examiner should accept that the term at issue is definite.

B. The Office Action states that claims 37-40 are allegedly vague and indefinite to the extent that these claims do not further limit the method of claim 26 which requires comparing FVD value of a test agent with control FVD value. Therefore, according to the Office Action, the method steps recited in claims 37-40 inherently much occur in order to determine FVD value.

The Examiner provides no specific basis for this assertion. In fact, one of skill would be aware of existing methods that can be used to perform the steps of claim 1 that are exemplified in claims 17-21. For example, alternatives would be to standardize the measured intensity of the test CAM to a control CAM. That is, use a control agent to get a standard baseline vessel density across a number of CAMS and compare the changes in density in CAMs used to test a substance of interest. Thus, it is not necessary to practice the methods of claim 17-21 in order to practice the present invention.

C. The Office Action further states that the term "lower spectrophotometer absorbance and higher spectrophotometer absorbance" in claim 41 is a relative term that allegedly renders the claims indefinite. It is allegedly unclear how much lower or higher absorbance would be considered to have inhibitory or stimulatory effects.

Applicants respectfully disagree and point out that the claim simply states that a lower spectrophotometric absorbance value of the test region of interest as compared to the spectrophotometric absorbance value of the control region of interest is indicative of the test molecule being useful as an inhibitor of angiogenesis, and that a higher spectrophotometric absorbance value of the test region of interest as compared to the spectrophotometric absorbance value of the control region of interest is indicative of the test molecule being useful as a stimulator of angiogenesis. Thus, Applicants believe it is clear that if the absorbance value of the test region of interest is lower, or less than the absorbance value of a control region, this is indicative of a test molecule being useful as an inhibitor of angiogenesis. Similarly, if the absorbance value of the test region of interest is higher, or greater than the absorbance value of a control region, this is indicative of a test molecule being useful as a stimulator of angiogenesis. All that is required is that the spectrophotometric absorbance of the test region be lower or higher than that of a control region in order to make a determination. The degree to which this occurs is not relevant for making this determination. Thus, Applicants believe that one of skill in the art would readily understand what is meant by these terms in claim 41.

Rejections Under 35 U.S.C. § 103(a)

A. The Office Action states that claims 26-34 and 36-40 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Brooks et al. (*Science*, 1994, 264, 570-571 IDS) and Robert et al. (*Cancer Res.* 1992; 52(4): 924-30) and Kimel et al. (*SPIE*, 1996, 2628, 69-76, IDS). According to the Office Action, "Brooks et al. teach the quantitation of angiogenic or anti-angiogenic activity by removing the filter disc and associated CAM tissue that is snap frozen and sections that are stained with different antibody for staining of vessel which is analyzed by using confocal microscopy. Brooks et al. analyzed

average rhodamine fluorescence for each vessel per unit area to measure laser confocal image to determine angiogenic or anti-angiogenic activity." Further stated in the Office Action is that Robert et al. provide evidence that photosensitizers that are preferentially retained by tumors have a selective affinity for proliferating neovasculature. According to the Office Action, Kimel et al. taught an *in vivo* uptake of porphyrin using CAM model to document fluorescence in real time at different time intervals to demonstrate biodistribution of porphyrin. Therefore, the Office Action concludes that in view of the teachings of Brooks et al. and Robert et al., it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method to measure angiogenic or antiangiogenic activity in a CAM assay of Brooks et al. by measuring fluorescent vascular density taught by Robert/Kimel with a reasonable expectation of success. One of ordinary skill in the art would have allegedly been sufficiently motivated to make such a modification, as Robert et al. had already disclosed that phthalocyanine and tetraphenyl porphine sulfonate compounds possess the greatest affinity for proliferating neovasculature relative to nonvascular tissue (supra) and particularly since both Brooks et al. and Kimel et al. sought to quantitate retention of fluorescent moiety in CAM. Although Brooks et al. did not administer the fluorescent moiety before removing the substrate, according to the Office Action, he generally embraced the potential of measuring fluorescence for quantification to better measure and compare angiogenesis. In addition, the Office Action alleges that Robert et al. and Kimel et al. provided motivation of using porphyrin or cyanine to measure the biodistribution in neovasculature in CAM assay.

Applicants would like to point out that the claimed methods are screening methods that allow the identification of compounds that possess angiogenic or antiangiogenic effects. As shown in Comparative Example 1, on pages 21-22 of the specification, it is Applicants' invention that provides a CAM assay that is superior to previous detection methods in the art. As indicated by the results set forth in Tables 2-4, when filter discs were evaluated by blinded observers using a detection method of the prior art, none of the three independent, blinded observers could visually appreciate differences between the groups when the methodology of blinded grading was applied.

However, as indicated in Example 1, differences were seen when an FVD value was assigned to each group. Thus, Applicants have provided novel CAM methods of measuring angiogenic and antiangiogenic activity that significantly improve the quantitation of these activities.

Applicants respectfully point out that none of Brooks et al., Kimel et al. or Roberts et al., either alone or in combination disclose or suggest a screening method to identify compounds with angiogenic or antiangiogenic activity. Brooks et al. administered known angiogenic inducers (bFGF and TNF-α) in order to study the effects of these known angiogenic agents on the expression of β_3 and β_1 integrins. This is a different goal to be achieved than assessing the angiogenic or antiangiogenic effects of a test compound via the claimed methods. The goal of screening is what motivates the nature and order of the steps in the claimed methods. This goal is lacking in any of the cited references, either alone or in combination.

Brooks et al. were studying the role of $\alpha_v\beta_3$ and β_1 integrins in the process of blood vessel formation. Thus, they analyzed expression of $\alpha_v\beta_3$ and β_1 integrins by laser confocal image analysis of CAM cryostat sections stained with fluorescently labeled monoclonal antibodies after removal of the CAM. The fluorescence values obtained corresponded to the amount of $\alpha_v\beta_3$ and β_1 expression during angiogenesis and was not a measure of angiogenesis itself. The research and results disclosed by Brooks et al. are not suggestive of using any CAM-based assay to screen for angiogenic or anti-angiogenic compounds. They were just studying a physiological/anatomical process. For the same reasons, their teaching is not suggestive of studying the unknown activity of an uncharacterized molecule (test molecule) using a test region from the CAM in which a test compound and a fluorescent labeled particle have been administered to a vessel and allowed to travel through each vessel contained in the test region of interest, such that the fluorescent vascular density (angiogenesis) of the test region of the CAM can be determined. Likewise, there is nothing in Brooks et al. that would motivate the administration of a fluorescent molecule after the administration of a test molecule and prior to the removal of the test region of the CAM, or that would provide a reasonable expectation that this would allow quantification of fluorescent vascular density.

With respect to Roberts et al., the focus of this paper was the study of the movement and retention of photosensitizers. The research and results disclosed by Roberts et al. are not suggestive of measuring the angiogenic or anti-angiogenic activity of any unknown test molecules. They merely provided information on how fluorescent molecules can be used in the study of a physiological/anatomical process. Although this reference indicates that photosensitizers are preferentially retained by tumors having a selective affinity for proliferating vasculature, these studies were conducted in mice or in CAM that was homogenized. In fact, in order to determine the photosensitizer content in the CAM of chicken embryos, according to Roberts et al., "[p]ossibly due to the fragile nature of the tissues, the following method resulted in optimal photosensitizer recovery and was used for all compounds. Chick tissues were homogenized in 0.1N NaOH (3ml/0.5 g of tissue) and centrifuged at 15,000g for 20 min (32). The supernatant was analyzed for photosensitizer content in a spectrofluorimeter." (see Roberts et al. page 925, paragraph 4). Applicants also note that Roberts et al. utilized 10 to 20 chick embryos for each data point (see Roberts et al., page 925, paragraph 3). Roberts et al. teaches that the distribution of a fluorescent particle in the vessels of the CAM cannot be accurately measured without homogenization of the CAM. Therefore, there is nothing in Roberts et al. that would motivate one of skill in the art to conduct screening of unknown compounds in general, nor is there any motivation or likelihood of success using a photosensitizer in the method of Brooks et al. because Roberts et al. teaches intact CAM does not work.

With regard to Kimel et al., they do not teach a method screening for angiogenic or anti-angiogenic compounds. This reference measured the *in vivo* uptake of natural porphyrins (fluorescent molecules) which is a factor in photodynamic therapy. There is no disclosure in this paper directed to screening for any activity of unknown molecules (test compounds). Thus, there is no motivation to use any steps of the disclosed method for the purpose of measuring angiogenic or anti-angiogenic activity of an unknown compound. Furthermore, although the study was conducted in CAM, the results showed an inconsistent or unstable pattern of fluorescence that rendered it unhelpful for determining angiogenic activity of a compound. More specifically, in measuring the

uptake of uroporphyrin III (UP), coproporphyrin III (CP) and protophorphyrin IX (PP), this reference shows that intravascular fluorescence for protophorphyrin IX (PP) was low and dominated by autofluorescence (see Kimel et al., page 70). For CP, the vessel becomes highly fluorescent for about 20 minutes after injection. At later times, the fluorescence of the surrounding CAM tissue (F_{matrix}) increases, whereas the $F_{intravascular}$ decreases, due to extravasation of the sensitizer (see Kimel at l., page 71 and Figure 5). For UP, a gradual increase of $F_{intravascular}$ could be observed up to 20-30 minutes after injection followed by concomitant increase of F_{matrix} (see Kimel et al., Figure 6). Therefore, there is no indication in Kimel et al. that fluorescent labeled particles (e.g. porphyrins) can be utilized to obtain a consistent image or level of fluorescence in the vessels of the CAM. Because consistency and stability of fluorescence are crucial to the step of removing a test region of the CAM in order to accurately assess fluorescence vascular density, Kimel et al. teaches away from the claimed method. Therefore, one of skill in the art would not be motivated to utilize the fluorescent particles of Kimel et al. in the method of Brooks et al. in order to measure angiogenesis or antiangiogenesis. Even if one of skill in the art were motivated to do so, there was no reasonable expectation that the useful, real-world results obtained by Applicants method could be achieved. Therefore, it would not have been obvious for one of skill in the art to combine Brooks et al. with Roberts et al. and/or Kimel et al. to arrive at the present invention. Thus, Applicants believe the present rejection has been overcome and respectfully request its withdrawal.

B. Claims 26-40 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Brooks et al; (*Science*, 1994, 264, 570-571 IDS) and Rizzo et al. (*Microvascular Res.* 1995, 49, 49-63, IDS). According to the Office Action, Rizzo et al. taught a method to quantitate the relative microvascular permeability associated with tumorigenesis and normal angiogenesis by microinjecting a graded series of FITC-dextrans into a vessel of CAM and then measuring the fluorescence by a confocal attachment to differentiate different capillary networks. According to the Office Action, in view of the teachings of Brooks et al. and Rizzo et al., it would have been obvious for

one of ordinary skill in the art, at the time the claimed invention was made, to modify the method to measure angiogenic or antiangiogenic activity in a CAM assay of Brooks et al. by measuring fluorescence vascular density taught by Rizzo et al. with a reasonable expectation of success.

.Applicants respectfully point out that Rizzo et al. relates to a method of studying microvascular permeability to macromolecules during normal angiogenesis. Because this art focused on the use of known molecules to study a physiological/anotomical response, there is no suggestion of doing any screening of unknown compounds. Thus, there is no motivation in Rizzo et al. to do anything related to screening. Furthermore, to accomplish this goals of its study, Rizzo et al. microinjects FITC-dextran into a shell-less CAM preparation. In other words, the embryo is removed from the shell and cultured, prior to administration of FITC-dextran to the CAM. Thus, the experimental approach used by Rizzo et al. differs significantly from the claimed method. No test molecule is administered and Rizzo et al. is merely studying the permeability of a fluorescent molecule during angiogenesis. No angiogenic effect attributable to any compound is being measured. In the methods of the present invention, the test molecule and the fluorescent labeled particle are administered to a test region in the CAM inside the shell. Nothing in Rizzo et al. would lead one of skill in the art to believe that a test molecule and FITC-dextran can be administered to a vessel in a test region of the CAM of a live embryo and that this test region can subsequently be removed for image capturing and quantitative analysis of angiogenic or antiangiogenic activity. Thus, Rizzo et al. provides no reasonable expectation of success. Furthermore, there is nothing in Rizzo et al. that would lead one of skill in the art to administer a test molecule as they don't teach a screening method. There is also no indication in Rizzo et al. that an assay system that differs from the one disclosed, as applicant's does, would provide any better or different results. Therefore, Rizzo et al. provides no motivation for one of skill in the art to substitute the fluorescence detection method of Brooks et al. with a fluorescence detection method that involves administering the fluorescent labeled particle to the CAM, inside the shell, prior to removal of the CAM for subsequent analysis. Thus, it would not be obvious to combine the teachings of Brooks et al. with Rizzo et al. in order to arrive at

the claimed invention. Such a combination would, in any case, not produce the claimed method. Therefore, Applicants believe this rejection has been overcome and respectfully request its withdrawal.

C. The Office Action states that claims 26-50 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Brooks et al.; (*Methods in Molecular Biology*, 129, 257-259, IDS); Kurz et al. (*Developmental Dynamics*, 1995, 203, 174-186) and Frasca et al. (*Oncogene*, 2001: 20, 3845-3856) as evidenced by Kinnman et al. (*Lab Invest.* 2001; 81(12): 1709-16).

Applicants respectfully point out that Kurz et al. is directed to the measurement of proliferation in the cultured (not intact) CAM. There is no teaching or suggestion in Kurz et al. of utilizing a metabolic agent to assess angiogenic or antiangiogenic activity of a test molecule. In fact, there is no accommodation of any test molecule in the method described by Kurz et al. The lack of teaching regarding screening makes this paper lack motivation to screen the activity of unknown compounds. Kurz et al. merely provides an alternative method of measuring proliferation that is carried out utilizing cultured CAMs, not CAMs in an eggshell as required by the present methods. There is no indication in Kurz et al. that BrdU can be administered to a vessel in a test region of the CAM, while still in the shell, such that the test region can subsequently be removed in order to measure proliferation. Thus, there is no reasonable expectation of success that a combination of Kurz et al. can be combined with Brooks et al. or Frasca et al. to produce a useful result.

Similarly, the results obtained by Frasca et al. pertain to the stimulatory effects of ST1571 on HGF induced migration of cancer cells in Matrigel, not a CAM. Thus, there is no teaching in Frasca et al. about the use of their system to measure an effect of any test molecule on angiogenesis. Frasca et al. describe the addition of XTT after the addition of a test molecule and described a method of measuring and comparing the metabolic activity at a specific wavelength, but this is not applied in the context of angiogenesis. Rather, it relates to the measurement of metabolic activity in cell proliferation that has no bearing on, and is, thus, not suggestive of the measurement of

angiogenic or anti-angiogenic activity in a test region of the CAM after administration of a test molecule and a metabolic agent *in vivo*.

With regard to Kinnman et al, this reference is directed to PDGF induced proliferation of hepatic stellate cells. The reference describes accomplishing its goal by studying the *in vitro* inhibition of PDFG-BB induced proliferation by ST1571, a known tyrosine kinase inhibitor. Because this method uses a known compound, it does not suggest the testing of any unknown compounds (test molecules). Thus it fails to motivate one of skill to perform any screening method for test molecules. The method is carried out in culture-activated HSC, not a CAM and measurement of same via BrdU assay, XTT assay and direct cell count. Once again, such generic references to methods of measuring proliferation by utilizing metabolic agents do not provide sufficient guidance or motivation for one of skill in the art to utilize these agents, as described in the claimed methods, in order to assess angiogenic or antiangiogenic activity of an unknown molecule. Therefore, there would be no motivation or any reasonable expectation of success that Brooks et al. in combination with any of Kurz et al., Frasca et al. and Kinnman et al. would allow one of skill in the art to arrive at the present invention. Thus, Applicants believe this rejection has been overcome and respectfully request its withdrawal.

Double Patenting

Claims 26-50 are provisionally rejected under 35 U.S.C. § 101 as allegedly claiming the same invention as that of claims 1-2, 7-22 and 27-35 of copending Application No. 11/014472. Applicant acknowledges the rejection and will formally respond to the provisional double patenting rejection in the appropriate application once claims are found to be allowable necessitating the removal of the provisional status of the rejection.

Pursuant to the above remarks, reconsideration and allowance of the pending application is believed to be warranted. The Examiner is invited and encouraged to

directly contact the undersigned if such contact may enhance the efficient prosecution of the application to issue.

A Credit Card Payment Form PTO-2038 authorizing payment in the amount of $1,020.00 is enclosed. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.

Gwendolyn D. Spratt
Registration No. 36,016

NEEDLE & ROSENBERG, P.C.
Customer Number 36339
(678) 420-9300
(678) 420-9301 (fax)

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8

I hereby certify that this correspondence, including any items indicated as attached or included, is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop AMENDMENT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date indicated below.

Gwendolyn D. Spratt — Date: 5-21-07

1: Int J Surg Investig. 1999;1(2):133-8.

Links

Image analysis for quantitation of solid tumor drug sensitivity.

Gibbs JF, **Slocum HK**, **Cao S**, **Rustum YM**.

Department of Surgical Oncology, Roswell Park Cancer Institute, State University of New York at Buffalo, 14263, USA.
jgibbs@sc3101.med.buffalo.edu

BACKGROUND: A method of assessing chemosensitivity of tissue has been described by Rotman et al. The aim of this study was to use image analysis to provide a more rapid and quantitative means of assessing drug effect on tissue proliferative capacity. METHOD: Fluoropyrimidine sensitive Ward rat colon adenocarcinoma tumor was implanted onto collagen impregnated cellulose fibers suspended on metal grids at an air-fluid interface and kept in a 95% air/5% CO2 incubator at 37 degrees C. The fluorescent microscopic image captured by a silicon intensified target (low light detecting) camera and linked to an image processing unit was measured for fluorescent brightness and tumor image area. Blinded 5-Fluorouracil (5-FU) drug treatment was begun 8 days after tumor explantation on the collagen-cellulose matrix. Tumor image area and fluorescent brightness were measured at 24 h pretreatment, 48 h posttreatment, and at 48 h post drug removal. RESULTS: Nontreated tumor cultures demonstrated an increase in area and fluorescent brightness with time following tumor implantation on the collagen gel. Dose responsiveness was seen with increasing concentrations of 5-FU. At the highest clinically achievable concentration of 5-FU (500 microM), there was a 39% decrease in area compared with the nontreated group, 113%. Linear dose responsiveness was not demonstrated between 50 and 150microM 5-FU. CONCLUSIONS: Fluoropyrimidine activity was demonstrated with the implemented image analysis system. The in vitro tumor sensitivity to FU using collagen gel was consistent with responsiveness of tumors in vivo borne by rats.

PMID: 11341633 [PubMed - indexed for MEDLINE]

Exhibit "B"

1: Cytometry. 1987 Nov;8(6):632-6.

Links

Calibration of flow cytometric fluorescence standards using the isoparametric analysis of ligand binding.

Chatelier RC, **Ashcroft RG**.

Melbourne Tumour Biology Branch, Ludwig Institute for Cancer Research, Victoria, Australia.

To extract ligand-cell binding parameters from flow cytometric fluorescence data, one needs a method of converting the measured cellular fluorescence to the actual number of bound molecules producing that fluorescence. Bead standards containing a known number of fluorophores do not necessarily provide the correct conversion factor. We therefore present a method for calibrating flow cytometric bead standards. The technique uses the isoparametric analysis (Chatelier et al: EMBO J 5:1181-1186, 1986) to construct a plot of fluorescence per cell versus the number of bound ligands per cell, thus allowing a direct comparison of the quantum yields of the bead-associated fluorophore with that of the cell-bound fluorophore. The potential of this analysis is demonstrated by contrasting the fluorescent brightness of fluorescein isothiocyanate associated with thymocyte nuclei and synthetic polymer beads with that of fluoresceinated epidermal growth factor bound to A431 cells. When the standards are improperly calibrated, then the number of ligand-binding sites is incorrectly reported, and the derived Scatchard plot may exhibit an apparent positive or negative cooperativity as well as a strong dependence on cell concentration.

PMID: 3123175 [PubMed - indexed for MEDLINE]

Exhibit "C"

1: Zhonghua Zhong Liu Za Zhi. 1985 Nov;7(6):420-2.

Links

[Estrogen receptor (ER) status of breast cancer cells. I. Classification of breast cancer cells bound with fluoresceinate-estrogen]

[Article in Chinese]

Zheng S.

Based on the location and intensity of fluoresceinate-estradiol or 17-fluoresceinate-estrone bound to breast cancer cells, the estrogen receptor (ER) status of breast cancer cells were classified into A. B. C. D and E types. The type A was the entire cell marked with fluorescent brightness, B was nucleus marked with fluorescence but not the cytoplasm, C was cytoplasm but not the nucleus, D was only the nucleolus concentrated with fluorescence and type E was very faint fluorescence presented in the entire cell. Types A, B and C were taken as positive, and the others negative. Twenty human breast cancer cells bound with fluoresceinate-estradiol were evaluated by computer image processing technique and it was demonstrated that each of the five types of cells could be identified. The instant and contiguous observation after dropped the 17-fluoresceinate-estrone demonstrated the simultaneous presence of all these five types of cells. The breast cancer cells bound with fluorescence light the distribution of ER within a cell and, hence, the possibility of morphologic and biologic investigation of ER.

PMID: 3915457 [PubMed - indexed for MEDLINE]

Related Links

[Estrogen receptor (ER) of breast cancer cells. II. Quantitative analysis of breast cancer cells bound with fluoresceinate-ER] [Zhonghua Zhong Liu Za Zhi. 1986]

Tocotrienols inhibit the growth of human breast cancer cells irrespective of estrogen receptor status. [Lipids. 1998]

Evaluation of the pathologic and prognostic correlates of estrogen receptors in primary breast cancer. [Ann Surg. 1982]

Differential recruitment of coregulator proteins steroid receptor coactivator-1 and silencing mediator for retinoid and thyroid receptors to the estrogen receptor-estrogen response element by beta-estradiol and 4-hydroxytamoxifen in human breast cancer cells. [J Clin Endocrinol Metab. 2004]

[Estrogens, cathepsin D and metastasis in cancers of the breast and ovary: invasion or proliferation?] [C R Seances Soc Biol Fil. 1998]

See all Related Articles...

Exhibit "D"

1: Biochem Biophys Res Commun. 1999 Oct 22;264(2):556-60.

A novel mutant of green fluorescent protein with enhanced sensitivity for microanalysis at 488 nm excitation.

Ito Y, **Suzuki M**, **Husimi Y**.

Department of Functional Materials Science, Saitama University, Urawa, 338-8570, Japan.

Green fluorescent protein (GFP) has been utilized as a powerful reporter of gene expression and protein localization in cells. We discovered a mutant carrying point mutation S208L from a UV-excitable GFP (F99S/M153T/V163A). It had the enhanced fluorescence intensity. Introduction of the red-shifted mutations (F64L/S65T) to this mutant led to the GFP having the brightest mutants reported which were expressed in Escherichia coli and excited at 488 nm. The relative fluorescence intensities to that of wild-type GFP and GFPuv were increased about 120- and 10-fold, respectively. It was shown that the S208L mutation contributes to both a higher intrinsic brightness of GFP and a higher expression level in E. coli. Copyright 1999 Academic Press.

PMID: 10529401 [PubMed - indexed for MEDLINE]

Exhibit "E"

1: Langmuir. 2007 May 8;23(10):5563-70. Epub 2007 Mar 31.

Quenching of molecular fluorescence on the surface of monolayer-protected gold nanoparticles investigated using place exchange equilibria.

Nerambourg N, **Werts MH**, **Charlot M**, **Blanchard-Desce M**.

Synthese et Electrosynthese Organiques (UMR6510), CNRS, Universite de Rennes 1, Campus de Beaulieu, Bat. 10A, F-35042 Rennes Cedex, France.

The insertion of fluorescently labeled thiols into the protecting self-assembled monolayer on the surface of gold nanoparticles through place exchange reactions and the effects of this insertion on the photophysical properties of the fluorophores are investigated. Analysis of solution-phase fluorescence data using a dynamic equilibrium model yields the equilibrium constant for the place exchange equilibrium, as well as the relative fluorescence brightness of the fluorophores on the particle surface. In all cases we find a significant quenching of the fluorescence, and potential reasons for this quenching are discussed. In the case of these relatively small particles (4.5 nm diameter), the quenching appears to be mainly related to enhanced nonradiative deactivation pathways. The place exchange equilibrium constant reveals a reduced affinity of the fluorescently labeled thiols for insertion into the nonfluorescent alkylthiol monolayer (Keq approximately 0.2) compared to unlabeled alkylthiols.

PMID: 17397202 [PubMed - in process]